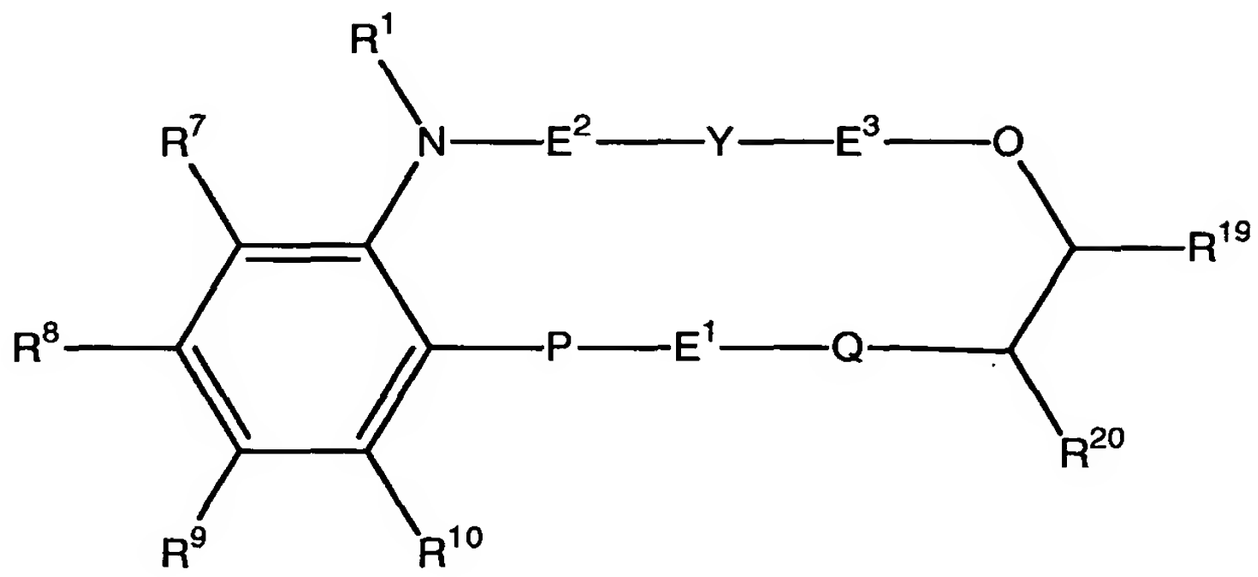
CLAIMS

WHAT IS CLAIMED IS:

1. A crown ether chelating compound having formula:



wherein

Y is O, S, NR^4 or is absent, wherein R^4 is selected from the group consisting of H, -L-R_X, -L--S_C, -L-DYE, C_1-C_{18} alkyl, aryl and heteroaryl ring system, which alkyl or ring system is optionally substituted by halogen, azido, nitro, nitroso, amino, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy that is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; wherein

R^{15} is selected from the group consisting of H, C_1-C_6 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive group;

each S_C is independently a conjugated substance;

each DYE is independently a reporter molecule;

P and Q are independently O, S or NR^3, wherein each R^3 is independently H or C_1-C_6 alkyl;

E^1, E^2, and E^3 are independently $-(CR^5_2)_n-$, $-(C(O)CH_2)_n-$, $-(CR^5_2)_nO(CR^5_2)_n-$ or E^2 is absent, where n = 2, 3 or 4, and each R^5 is independently H or CH_3, or two R^5 moieties on adjacent carbons of one or more of E^1, E^2 or E^3, when taken in combination, form a 5- or 6-membered aliphatic ring;

R^1 is selected from the group consisting of -L-R_X, -L-S_C, -L-DYE, C_1-C_{18} alkyl and C_7-C_{18} arylalkyl, each of which is optionally substituted by halogen, azido, nitro, nitroso, amino, hydroxy, cyano, C_1-C_6 alkoxy, an aryl or heteroaryl ring system, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, $-(C=O)-NR^{17}R^{18}$, C_1-C_6 alkylamino, C_2-C_{12} dialkylamino, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino (-$NR^{17}R^{18}$), hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$ or $-(C=O)-NR^{17}R^{18}$;

R^{19} and R^{20} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo moiety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, $-(SO_2)-R^{15}$, $-(SO_2)-O-R^{15}$, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, taken in combination, form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, $-(C=O)-R^{15}$, $-(C=O)-O-R^{16}$, or $-(C=O)-NR^{17}R^{18}$;

or any two adjacent substituents R^7-R^{10}, or R^{19} and R^{20}, taken in combination with each other, form a fused DYE.

2. The compound according to Claim 1, wherein said P, Q and Y are O.

3. The compound according to Claim 2, wherein said E^1, E^2, and E^3 are each $-(CR^5{}_2)_n$ wherein R^5 is H and each n is 2.

4. The compound according to Claim 3, wherein at least one of said R^1, R^7, R^8, R^9, R^{10}, R^{19} or R^{20} is -L-Rx, -L-Sc or -L-DYE or R^8 in combination with R^9 form a fused DYE.

5. The compound according to Claim 4, wherein said L is a single covalent bond, or a covalent linkage that is linear or branched, cyclic or heterocyclic, saturated or unsaturated, having 1-20 nonhydrogen atoms selected from the group consisting of C, N, P, O and S; and are composed of any combination of ether, thioether, amine, ester, carboxamide, sulfonamide, hydrazide bonds and aromatic or heteroaromatic bonds.

6. The compound according to Claim 4, wherein said -Rx is selected from the group consisting of an acrylamide, an activated ester of a carboxylic acid, a carboxylic ester, an acyl azide, an acyl nitrile, an aldehyde, an alkyl halide, an anhydride, an aniline, an amine, an aryl halide, an azide, an aziridine, a boronate, a diazoalkane, a haloacetamide, a halotriazine, a hydrazine, an imido ester, an isocyanate, an isothiocyanate, a maleimide, a phosphoramidite, a reactive platinum complex, a silyl halide, a sulfonyl halide, a thiol and a photoactivatable group.

7. The compound according to Claim 6, wherein said –Rx is selected from the group consisting of carboxylic acid, succinimidyl ester of a carboxylic acid, hydrazide, amine and a maleimide.

8. The compound according to Claim 4, wherein said –Sc is selected from the group consisting of an amino acid, a peptide, a protein, a polysaccharide, a nucleoside, a nucleotide, an oligonucleotide, a nucleic acid, a hapten, a psoralen, a drug, a hormone, a lipid, a lipid assembly, a synthetic polymer, a polymeric microparticle, a biological cell or a virus.

9. The compound according to Claim 8, wherein said –Sc is selected from the group consisting of an antibody or fragment thereof, an avidin or streptavidin, a biotin, a blood component protein, a dextran, an enzyme, an enzyme inhibitor, a hormone, an IgG binding protein, a fluorescent protein, a growth factor, a lectin, a lipopolysaccharide, a microorganism, a metal binding protein, a metal chelating

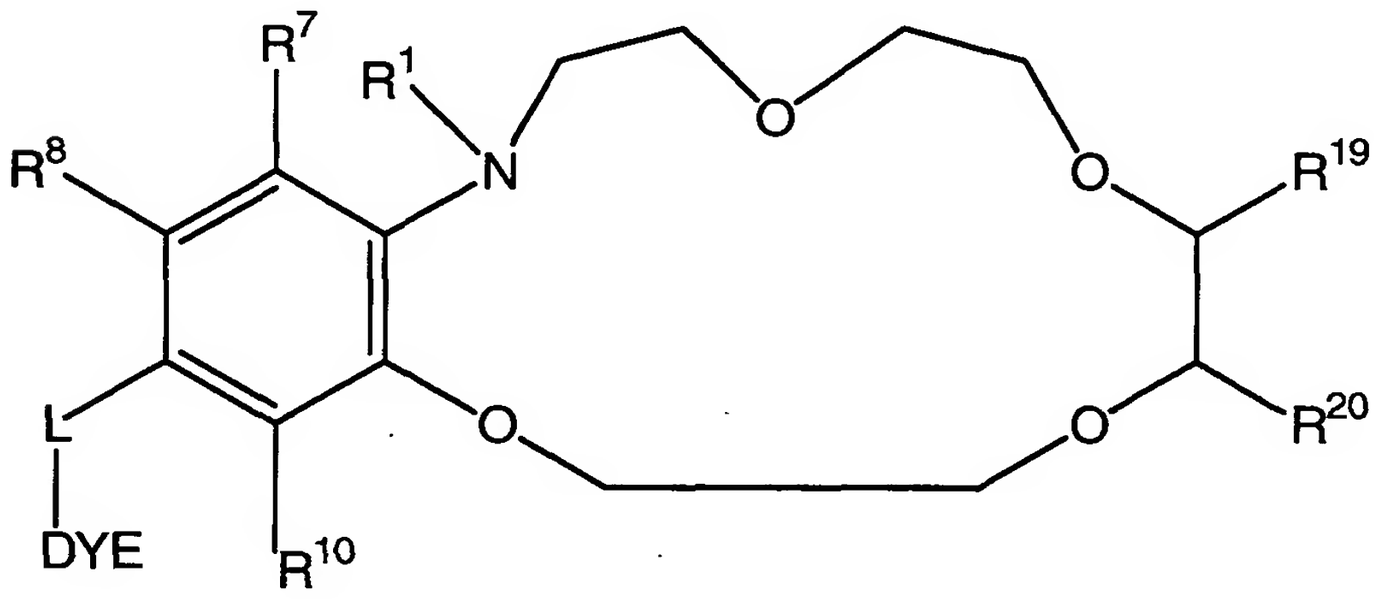
moiety, a non-biological microparticle, a peptide toxin, a phosphotidylserine-binding protein, a structural protein, a small-molecule drug, or a tyramide.

10. The compound according to Claim 5, wherein said –DYE is selected from the group consisting of xanthene, borapolyazaindacene, carbocyanine, benzofuran, quinazolinone, indole, a benzazole, oxazine, and coumarin.

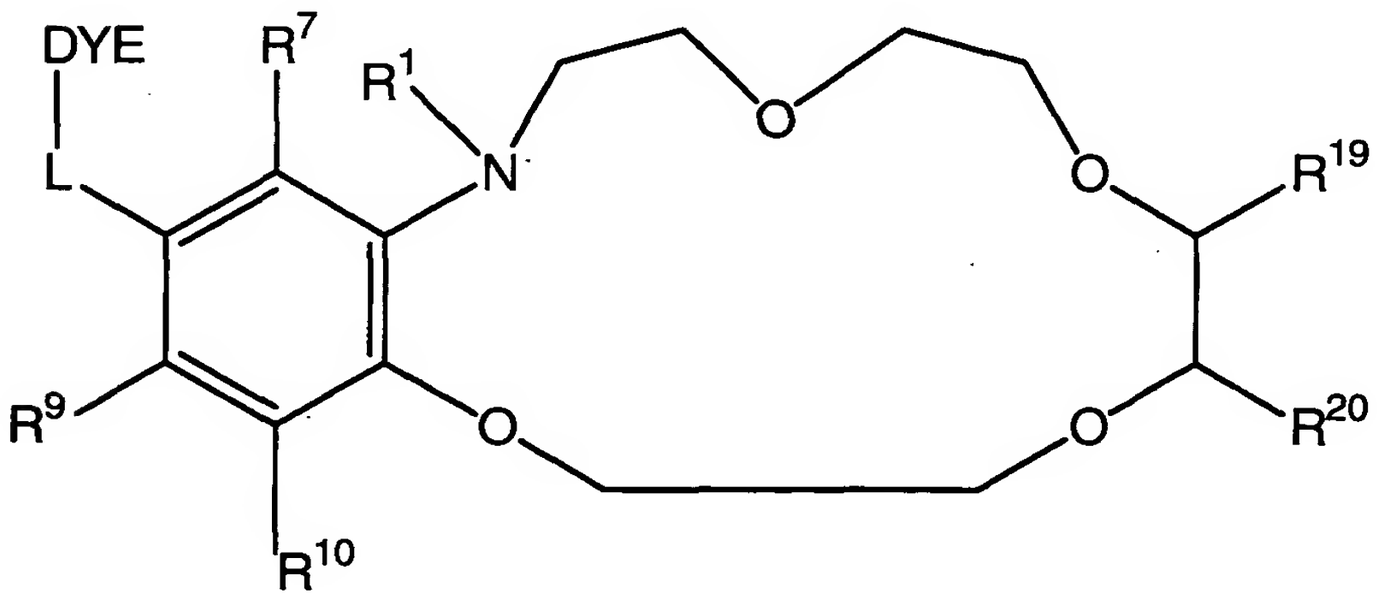
11. The compound according to Claim 10, wherein said –DYE moiety is independently substituted by a lipophilic group.

12. The compound according to Claim 11, wherein said lipophilic group is an AM or acetate ester.

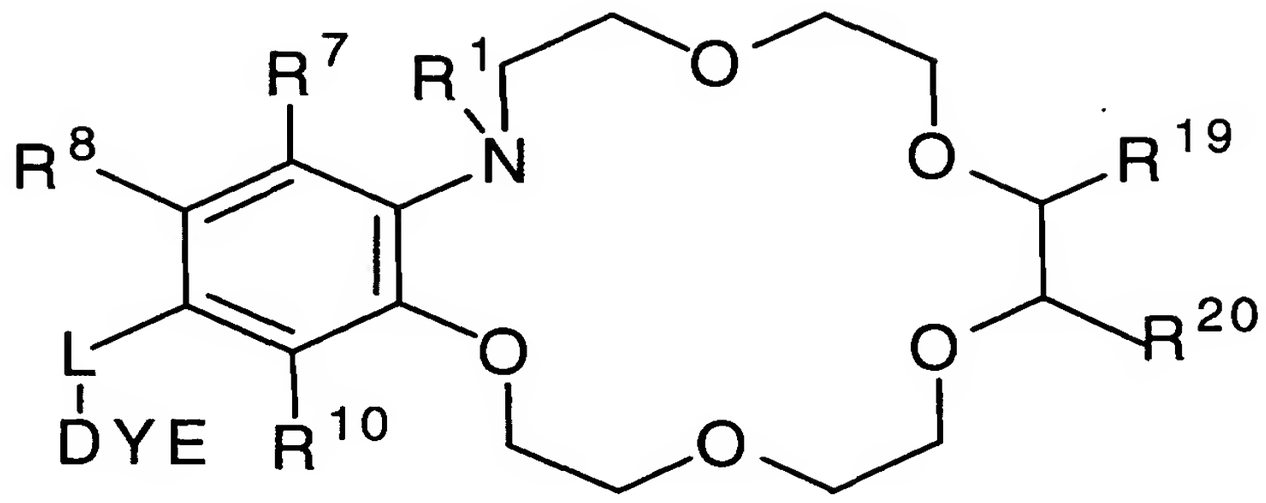
13. The compound according to Claim 1, wherein said compound is selected from the group consisting of



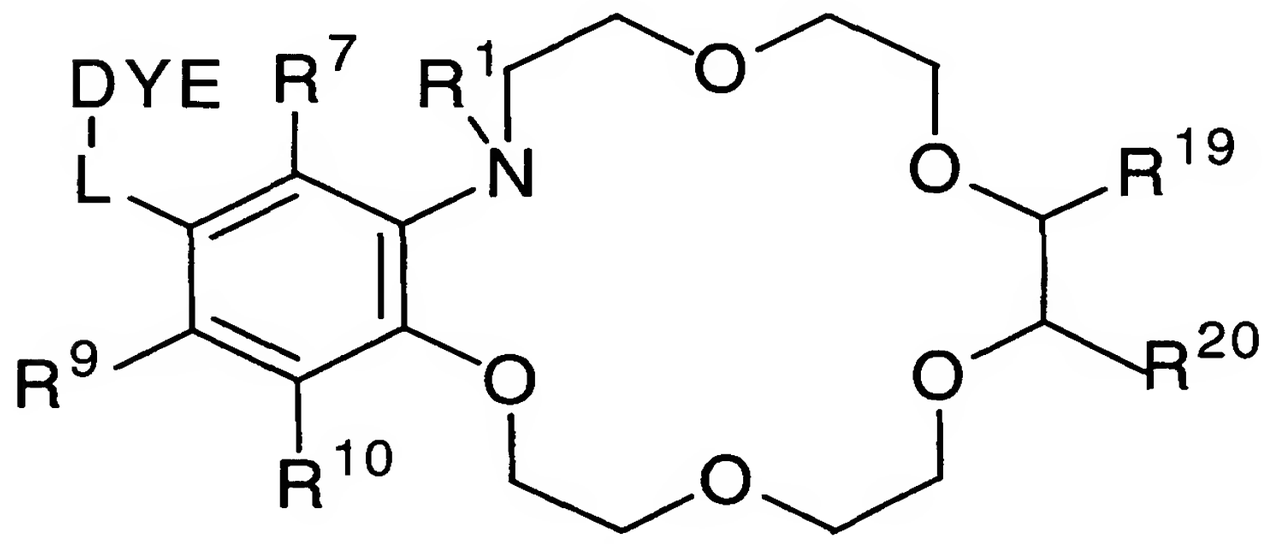
Formula (II)(a),



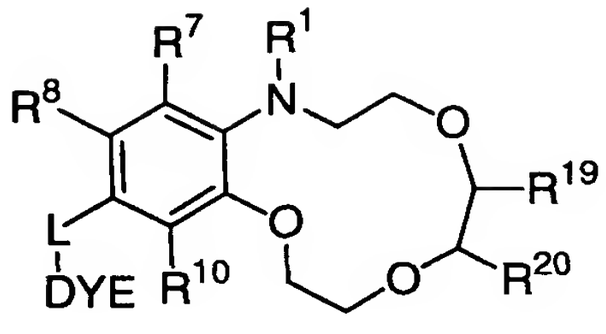
Formula (II)(b),



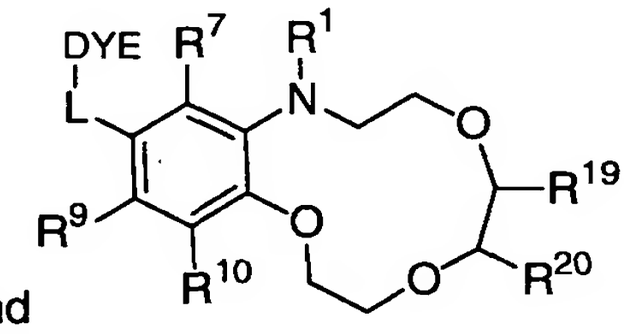
Formula (II)(c),



Formula (II)(d),



Formula (II)(e) and



Formula (II)(f).

14. The compound according to Claim 13, wherein said DYE is selected from the group consisting of borapolyazaindacene, xanthene and indole.

15. The compound according to Claim 13, wherein said DYE moiety is independently substituted by a lipophilic group.

16. The compound according to Claim 15, wherein said lipophilic group is an AM or acetate ester.

17. The compound according to Claim 13, wherein R^7, R^8, R^9, R^{10}, R^{19} and R^{20}, when present, are H.

18. The compound according to Claim 17, wherein R^1 is C_1-C_6 alkyl that is substituted one or more times by amino (-$NR^{17}R^{18}$), –(C=O)-O-R^{16} or –(C=O)-$NR^{17}R^{18}$.

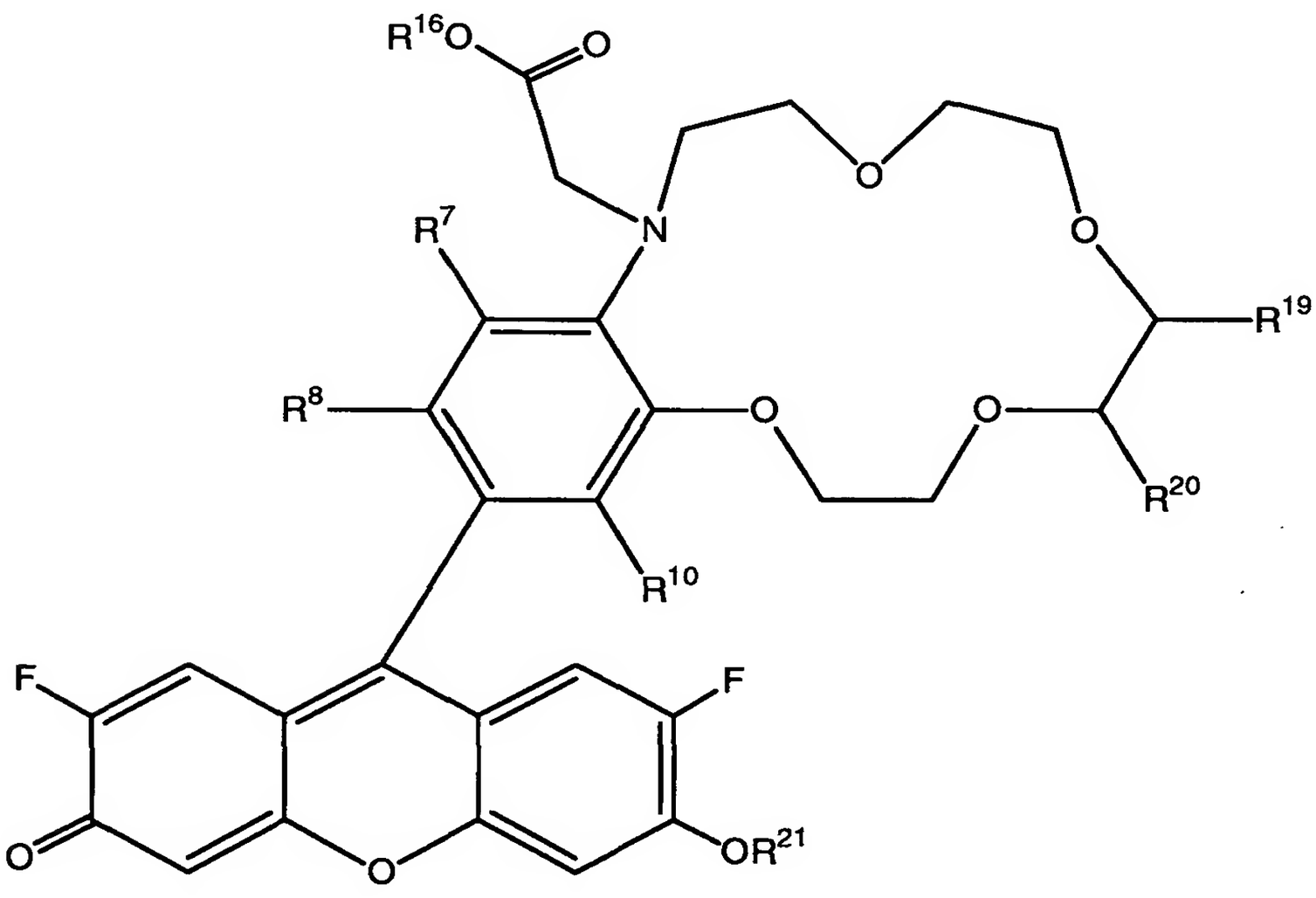
19. The compound according to Claim 18, wherein said R^1 is methyl or ethyl.

20. The compound according to Claim 19 wherein said R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, and a biologically compatible salt.

21. The compound according to Claim 20 wherein said R^{16} is methyl.

22. The compound according to Claim 18 wherein said R^{17} and R^{18} are each methyl.

23. A compound having formula:



wherein R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, and a biologically compatible salt;

R^{19} and R^{20} are selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16} and -(C=O)-$NR^{17}R^{18}$;

or R^{19} and R^{20} taken in combination form a fused six-membered benzo moiety that is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is itself optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

R^{15} is selected from the group consisting of H, C_1-C_6 alkyl, -L-R_X, -L-S_C and -L-DYE;

R^{16} is selected from the group consisting of H, C_1-C_6 alkyl, benzyl, a biologically compatible esterifying group, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE;

R^{17} and R^{18} are independently selected from the group consisting of H, C_1-C_6 alkyl, C_1-C_6 carboxyalkyl, alpha-acyloxyalkyl, trialkylsilyl, a biologically compatible salt, -L-R_X, -L-S_C and -L-DYE; or R^{17} and R^{18} taken in combination form a 5- or 6-membered aliphatic ring that optionally incorporates an oxygen atom;

each L is independently a covalent linkage;

each R_X is independently a reactive group;

each S_C is independently a conjugated substance;

each DYE is independently a reporter molecule;

R^7, R^8, and R^{10} are independently selected from the group consisting of H, halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl and C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(SO_2)-R^{15}, -(SO_2)-O-R^{15}, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$;

or R^7 taken in combination with R^8 form a fused six-membered benzo moiety, which is optionally substituted by halogen, azido, nitro, nitroso, amino, cyano, -L-R_X, -L-S_C, -L-DYE, C_1-C_6 alkyl or C_1-C_6 alkoxy, each of which is optionally substituted by halogen, amino, hydroxy, -(C=O)-R^{15}, -(C=O)-O-R^{16}, or -(C=O)-$NR^{17}R^{18}$; and,

R^{21} is selected from the group consisting of H, C_1-C_{18} alkyl, C_7-C_{18} arylalkyl and a lipophilic group each alkyl is optionally substituted by -(C=O)-R^{15}, -(C=O)-O-R^{16}, or C_1-C_6 alkoxy.

24. The compound according to Claim 23, wherein said R^7, R^8, and R^{10} are H.

25. The compound according to Claim 24, wherein said R^{19} and R^{20} are H.

26. The compound according to Claim 25, wherein said R^{16} is methyl or a biologically compatible esterifying group.

27. The compound according to Claim 23 wherein said R^{16}, R^{19} or R^{20} is –L-Sc.

28. A composition comprising:

a. a compound according to any one of Claims 1-27; and,
b. a metal ion that is capable of being chelated by said compound.

29. The composition according to Claim 28, wherein said metal ion is selected from the group consisting of Na^+, Li^+, K^+, Ca^+, Zn^+ and Rb^+.

30. A method for binding a target metal ion in a sample, comprising steps of:

a. contacting said sample with a metal chelating compound according to any one of Claims 1-27
b. incubating said sample and said metal chelating compound for sufficient time to allow said compound to chelate said target metal ion whereby said metal ion is bound.

31. The method according to Claim 30, wherein said method further comprises illuminating said metal chelating compound with a suitable light source whereby said target ion is detected with the proviso that at least one of R^1, R^4, R^7, R^8, R^9, R^{10}, R^{19} or R^{20} is –L-DYE or at least two of R^7-R^{10} or R^{19} and R^{20}, taken in combination, form a fused DYE.

32. The method according to Claim 31, wherein said target metal ion is selected from the group consisting of Na^+, Li^+, K^+, Ca^+, Zn^+ and Rb^+.

33. The method according to Claim 32, wherein said target metal ion is Na^+.

34. The method according to Claim 32, wherein said sample comprises living cells, cellular components, proteins, peptides, buffer solutions or biological fluids.

35. A method for binding and detecting target ions in a live cell, said method comprises:

a) contacting a sample of live cells with a crown ether compound according to any one of Claims 1-27 with the proviso that said compound comprise a DYE moiety and at least one lipohilic group;

b) incubating said sample and said crown ether chelate compound for sufficient time to allow said compound to chelate said target metal ion; and,

c) illuminate said sample with an appropriate wavelength whereby said target ion is detected in a live cell.

36. The method according to Claim 35, wherein said DYE moiety is substituted by a lipophilic group.

37. The methd according to Claim 36 wherein said lipophilic group is an AM or acetate ester.

38. A kit for binding a metal ion in a sample, comprising:
a compound according to any one of Claims 1-27; and, comprising one or more components selected from the group consisting of a calibration standard of a metal ion, an ionophore, a fluorescent standard, an aqueous buffer solution and an organic solvent.